PRODUCTION OF OPTICALLY PURE (S)-IBUPROFEN ACID VIA ENZYMATIC DYNAMIC KINETIC RESOLUTION IN HOLLOW FIBER MEMBRANE REACTOR

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ABSTRACT

(S)-Ibuprofen acid is classified as a non-steroidal anti-inflammatory drug (NSAID) that is well-known to be useful in treating mild to moderate pain, fever and inflammation. The production of this optically pure compound poses a major challenge for the pharmaceutical industry. In the present technology, a hollow fiber membrane reactor (HFMR) system is innovatively designed to carry out dynamic kinetic resolution (DKR) of racemic Ibuprofen ester. DKR is described as a chiral separation technique that incorporates the classic kinetic resolution (KR) with in situ racemization. The operation principle of the HFMR is simple and environmental friendly. It combines the reaction, product separation and by-product recovery in a single unit. The design of the HMFR was clearly discussed in this article. The HFMR performance was studied by varying the important process parameters such as effect of alcohols, substrate and base concentration as well as the flow rates for both lumen and shell side. The HFMR gave product enantiomeric excess of 98.5% and 99% conversion at molar ratio of 1:3 with respect to the substrate and alcohol, 50 mM of base concentration, shell and lumen loop flow rates of 80 and 200 ml min⁻¹ respectively. The application of enzymatic biphasic membrane reactor for DKR provides a green and efficient way for obtaining the high purity product without any generation of by product.

INTRODUCTION

Ibuprofen acid are categorized as non-steroidal anti-inflammatory drugs (NSAIDs). They are widely used for treating mild to moderate pain, fever and inflammation. In a chiral environment, one enantiomer may display different chemical and pharmaceutical behavior than the other enantiomer. Usually, one isomer binds preferentially while the other has little or no activity. In ibuprofen racemates, majority of the therapeutic value of ibuprofen is contributed by the (S)-configuration as evidenced by the known higher activity exhibited by the (S)-enantiomers (Long et al., 2005). The (R)-enantiomer of drug will not necessarily behave the same way as the (S)-enantiomer of the same drug when taken by a patient. The difference in activity between the two enantiomers means that the (R)-enantiomer has little effect on the drug’s activity and does not cause any serious side effects (Fazlena et al., 2005). Apart from that, the crystal structure of (S)-ibuprofen acid was claimed to be inherently different than those in racemic form. (S)-Ibuprofen acid has shown different properties such as better solubility and dissolution rate than its racemic form (Hutt & Caldwell, 1984). This becomes an advantage for using a single active isomer of profens in preparing new drug formulation or
medicine. In the present work, our main goal is to eliminate the \((R)\)-Ibuprofen from racemic compound and obtain the desired product which is \((S)\)-Ibuprofen acid. Recently, enantioseparation of chiral compounds via resolution approach has been practiced by a number of researchers (Paál et al., 2008; Haak et al., 2008 and Xie et al., 2009). Enzymatic resolution has become a particularly attractive approach in the production of optically pure compounds due to the mild process condition and high product yield (Bhatia et al., 2004).

Two types of commonly used bio-catalytic reactors such as; batch bioreactor and enzyme-immobilized membrane reactor have been employed in the resolution of chiral drugs (Fazlena et al., 2005; Long et al., 2005; Lau et al., 2010). The behavior of hollow fiber membrane reactors were widely investigated from a theoretical point of view in the last 10 years (Lu et al., 2002; Calabrò et al., 2002; Long et al., 2003; Wang et al., 2004; Choi et al., 2007). Long et al. (2003) has analytically solved for reactant concentration profiles in finite hollow and solid cylinders, generating effectiveness-factor graphs allowing for both radial and axial dependence of the reactant concentration. Neither the skin nor the spongy matrix offer much diffusional resistance, and hence an enzyme reaction carried out in a hollow fiber device will usually be limited either by diffusion across the membrane fiber or reaction kinetics on the sponge-side (Calabrò et al., 2002). The mass transfer resistance offered by the tube-side stagnant liquid film and the fiber wall are negligible when compared to the cell suspension mass-transfer resistance, as justified by Long et al. (2003).

In a classic resolution, the product is formed at the very beginning of the reaction but decrease when the reaction runs at about 50% conversion. This depletion is caused by gradual disappearance of the faster reacting enantiomer from the racemic substrate mixture (Yuchun et al., 2000). On the other hand, the depletion of the desired enantiomer does not occur if substrate is constantly racemized throughout the course of the reaction. In other words, in dynamic resolutions the selection of the faster reacting enantiomer remains a simple task for the enzyme because it always faces a racemic substrate throughout the reaction. Thus the reaction does not come to a standstill and can be ran to completion by converting all of the racemic starting material into product P. Fig. 1 demonstrates that high activity lipase enzyme should be used to provide maximum catalysis capability in order to achieve high rate of \((S)\)-ester hydrolysis. When the \((R)\)-ester concentration builds up in the system, high efficient base catalyst should be used to provide rapid racemization of \((R)\)- and \((S)\)-esters. The rate of \((S)\)-ester hydrolysis should overtake the rate of racemization so that the high efficient DKR is achieved.

![Fig. 1: Schematic representation of a dynamic kinetic resolution (DKR). \((R)\)- and \((S)\)-represent the two enantiomers of the starting material; P and Q represent the two enantiomers of the product. Abbreviations: \(K_{\text{rac}}\) rate constant for racemization; \(k_R\) rate constant for reaction of \((R)\)- isomer; \(k_s\) rate constant for reaction of \((S)\)- isomer (Pamies & Backvall, 2004).](image-url)
In the present work, a lipase mediated hollow fiber membrane reactor was designed to de-racemize the racemic ibuprofen ester. The lipase was entrapped inside the spongy matrix of hydrophilic synthetic membranes which are made of polyacrylonitrile (PAN). The high surface-to-volume ratio of the hollow fiber membrane is an advantage for membrane reactor as it allows high biocatalyst density in a relatively small reactor volume. The hollow fiber membranes were assembled into a bundle of parallel tubes in a cylindrical cartridge. The selective barrier of the membrane wall separates the membrane reactor into two distinct compartments, namely luminal side and shell side. Both the substrate racemic ester and aqueous buffer flow respectively at shell and lumen sides during the operation. The hydrophilicity of the membrane prevents the organic substrate to mix with the aqueous phase. The product ((S)-ibuprofen acid) which is highly soluble in aqueous phase diffuses through the membrane and enters the lumen side (Lau et al., 2010). On the other hand, the unreacted substrate ester ((R)-ibuprofen ester) remained in the shell side after the hydrolysis process. Meanwhile, in-situ racemization of the (R)-ibuprofen ester occurs within the organic loop and recovers the unreacted substrate. The continuous racemization in the dynamic kinetic resolution will keep recirculating until the steady state is achieved and contributes to a 100% theoretical conversion and $ee_p$. The enzymatic hollow fiber membrane reactor system is depicted as diagram in Fig. 2.

![Fig. 2. The enzymatic membrane reactor system for the resolution of racemic ibuprofen ester](image)

**MATERIALS AND METHODS**

**Enzymes and Materials**

Lipase from *Candida rugosa* EC 3.1.1.3 (Type VII, 724 units per mg solids) was supplied by Sigma-Aldrich (MI). The lipase was used as purchased without further purification. BCA protein assay was obtained from Pierce (IL). (R,S)-ibuprofen acid 99% and (S)-ibuprofen acid 99% were purchased from Acros (Belgium). Isooctane 99%, Sodium dihydrogen phosphate ($\text{KH}_2\text{PO}_4$, MW 136.09), disodium hydrogen phosphate dehydrate ($\text{Na}_2\text{HPO}_4\cdot\text{2H}_2\text{O}$, MW
177.99) were supplied by Fisher Chemicals (UK). Other chemicals and reagents used were of analytical grade.

**Chemical Syntheses of Ibuprofen Ester**

Racemic 2-ethoxyethyl-ibuprofen ester was chemically synthesized in our laboratory from the racemic ibuprofen acid, and 2-ethoxyethanol using isooctane as co-solvent and p-toluenesulfonic acid as catalyst. The esterification was carried out using a reflux setup in a Dean and Stark apparatus for 8 h. After cooling at room temperature, the organic mixture was washed with 5% sodium hydroxide and water. The organic layer was dried using sodium sulfate and evaporated by a rotary evaporator (Buchi Model R-114, Switzerland). The residual was further purified by micro-distillation to remove impurities. The ester structure was determined by H-NMR (Bruker Model Advance 400, Swiss) and FTIR (Perkin-Elmer Model System 2000, Shelton). For synthesis of (S)-ibuprofen ester, (S)-ibuprofen acid was used in the reflux, other conditions remain the same.

**Enzyme Immobilization on Membrane Reactor**

2 g/L of *Candida rugosa* lipase solution was prepared using phosphate buffer at desired pH value. Enzyme immobilization on the membrane sponge layer was carried out by ultrafiltration of one liter lipase solution from shell side to fiber lumen with TMP of 35 kPa and flow velocity $1.5 \times 10^{-2}$ m/s at room temperature. The volume of the buffer collected from the permeate and retentate were recorded. After filtration of lipase solution, ultrafiltration was continued using 1 L of fresh buffer for washing. Residual water in shell side was completely filtered by purging with nitrogen gas. The washing solution in the permeate and retentate were collected for protein measurement. For lipase immobilized on the inner lumen, the same procedure was used except that the direction of ultrafiltration was from lumen to shell. The amount of protein (lipase) adsorbed onto the fibers of the hollow fiber membrane reactor was measured as the difference between the protein content of the lipase buffer solutions before and after the immobilization procedure. The protein concentration was quantified based on BCA standard test tube protocol (Pierce, Rockford, IL).

**Parameter Studies**

Performance of the EMR was determined by carry out the parameter studies. In this research, four parameters were taken into consideration. These parameters are effect of alcohols, substrate concentration as well as flow rates for both lumen and shell loop. In the experiments with varying substrate concentration, phosphate buffer solution of fixed pH value of 8 was used as the aqueous phase in the EMR process. Aqueous buffer of slightly alkaline was used for better extraction and dissolution of (S)-acid produced from the reaction.

For all other experimental runs, lipase solution of 2 g/L in 50 mmol L$^{-1}$ phosphate buffer pH 8 was used in the immobilization prior to enzymatic membrane reaction, the volume of the organic and aqueous in the feed tank were 5L and 10L, respectively, the recycle organic loop flow rate was maintained at 80 ml/min and recycle aqueous loop at 200 ml/min at TMP of 40 kPa and reaction temperature set at 40°C unless otherwise stated.

**Effect of Alcohols**

Esterification of racemic acid with several types of alcohols (2-ethoxyethanol, butan-1-ol and hexan-1-ol) will be carried out in the batch reflux reactor. The reactant molar ratio will be set to be 1:2.5 respect to racemic acid and alcohol, respectively. The mixture will be refluxed at
105°C for 8 hours. Then the ester produced by different alcohols will be collected in different tanks and ready for the hydrolysis in EMR unit.

**Substrate Concentration**

Experiments was carried out with substrate ester concentration in the range of 10, 20, 30, 50 and 80 mmol L\(^{-1}\) at 45°C and pH 8 at constant organic flow rate of 80 ml/min and aqueous flow rate of 200 ml/min.

**Aqueous Phase Flow Rate**

The recirculation rate of aqueous phase was studied for both cases of lipase immobilized on the sponge layer and inner lumen. The organic phase flow rate was in the range of 20-200 ml/min at fixed organic flow rate of 80 ml/min in the organic circuit for cases where lipase was immobilized on the spongy layer. 50 mmol L\(^{-1}\) phosphate buffer solutions at pH 8 will be used in all the experimental runs.

**Base Concentration**

The effect of base concentration was investigated by manipulating the concentration from 10 mM to 100 mM. 50 mmol L\(^{-1}\) phosphate buffer at flow rates of 200 ml/min will be carried out at fixed organic flow rate of 80 ml/min at pH 8.0.

**RESULT AND DISCUSSION**

**Effect of Substrate Concentration**

The result in Fig. 3 shows that the product enantiomeric excess, \(ee_p\), and conversion, \(X\), decreased with the increase of initial racemic substrate concentrations. This phenomenon was due to the occurrence of the enzyme inhibition by the uncompetitive inhibitor. The substrate \((R)-ibuprofen ester acts as an uncompetitive inhibitor in the hydrolysis of racemic ester (Long et al., 2005) and it tends to attack the allosteric sites and reduces the hydrolysis rate due to the changes of the enzyme conformation. Since the complex formed is reversible, it then further dissociates to form the products.

![Graph showing the effect of substrate concentration on ee, X at reaction time, t=240 min; base concentration, \(s_{base}=10\) mM; alcohol concentration, \(s_{alc}=50\) mM.]

On the other hand, by reducing the ester concentration in the organic phase, the chances of substrate inhibition are mitigated. The active sites bind easily with the \((S)\)-substrate and enhances the hydrolysis reaction. Meanwhile, the enzyme activity is maintained at a high
level. However, the equimolar properties of a unique racemic compound lead to a problem where the increased level of initial substrate concentration would also increase the amount of the uncompetitive inhibitor. This not only adds the uncompetitive inhibitor to the system, but eventually increases the amount of non-competitive alcohol after the hydrolysis. Hence, the \( ee_p \) value and conversion were reduced while the amount of unreacted substrate increased with an increasing amount of initial \((R,S)\)-ibuprofen ester concentration.

**Effect of Alcohols**

The effect of the non-competitive inhibition was investigated by manipulating the alcohol concentration in the organic phase. The result presented in Fig. 3 shows that higher amount of alcohol in the system gave lower substrate conversion and product enantiomeric excess. The process exhibited low productivity in the presence of alcohol when it bound to the enzyme-substrate complex and caused the active sites to be unavailable to the substrate. The hydrolysis of \((R,S)\)-ibuprofen ester gave 0.5 conversion and presumably, zero product \( (ee_p=0.01) \) at 200 mM alcohol concentration. On the other hand, it was also observed that high value of both conversion and \( ee_p, (X=0.99, ee_p=0.99) \) were obtained at the lowest alcohol concentration i.e. 20 mM.

![Fig. 3: Effect of alcohol concentration on \( ee_s, ee_p \) and conversion at reaction time, \( t =240\text{min} \); base concentration, \( s_{base}=50\text{mM} \); initial concentration, \( s_{T0}=80\text{ mM} \).](image)

An acceptable range of the alcohol present in the bulk substrate was also observed. The organic phase with 20 to 60 mM alcohol could still provide high conversion and \( ee_p \). This implies that the organic phase of the EMR should be controlled at the lowest alcohol concentration as to provide a maximum \((S)\)-ibuprofen acid productivity. However, the results show that the initial substrate concentration exhibited stronger inhibition effect than the alcohol concentration. The hydrolysis of ibuprofen ester gave very low \( ee_p \) and conversion i.e \( ee_p=0.12, X=0.41 \) at maximum initial substrate of 100 mM while similar \( ee_p=0.12 \) and \( X=0.56 \) were obtained only when the system contains 160 mM of alcohol.

**Effect of Flow rate**

The effect of the lumen loop flow rate was studied. It was observed in Fig. 4 that the lumen flow rate showed insignificant effect on the conversion and enantiomeric excess. There were very little changes among the \( ee_s, ee_p \) and conversion despite of high lumen flow rate. The \( ee_p \)
and conversion profiles remained with a small increase at 0.8 and 0.9 respectively. On the other hand, the \( ee_p \) profile decreased gradually from 0.1 to 0.08 with the increase of lumen flow rate. The reason is probably due to the batch-wise operation of the EMR as well as the continuous circulation of aqueous phase in the lumen side. Eventually, the process reaches steady state after four hours of continuous operation. In addition, the constant enzyme activity in the hydrolysis produces equal amount of product along the hollow fiber and diffuses slowly into the lumen side. Since the production of (S)-ibuprofen does not get affected much by the lumen flow rate, the EMR should then operate at low to moderate flow rate so as to reduce the energy consumption.

![Fig. 4: Effect of lumen flow rates on \( ee_p \), \( ee_s \) and \( X \) at reaction time, \( t=240 \) min at alcohol concentration, \( s_{alc}=50\) mM; base concentration, \( s_{base}=50 \) mM and initial concentration, \( s_{T0}=80 \) mM.](image)

**Effect of Base Concentration**

As depicted in Fig. 5, the trioctylamine concentration gave a very significant effect on the product yield where the \( ee_p \) and conversion obtained were 0.2 and 0.6 respectively at 10 mM trioctylamine. Both values increased rapidly until they reached a steady state at \( ee_p=0.9 \) and \( X=0.95 \). The steady state of the DKR maintained after achieving the concentration of 60 mM. However, the substrate enantiomeric excess remained at \( ee_s=0.04 \) regardless of any changes of the base concentration.

![Fig. 5: Effect of base concentration on \( ee_s \), \( ee_p \) and \( X \) at reaction time, \( t=240 \) min at alcohol concentration, \( s_{alc}=50\) mM; initial concentration, \( s_{T0}=80 \) mM.](image)
This implies that the DKR is sufficient to provide high ee and conversion at moderate base concentrations even with high initial substrate concentration i.e. $s_{T0}=80$ mM. The base concentration is vital in controlling the dynamic equilibrium among ($R$,$S$)-esters and enolate molecules. In addition, the literature also reported that the rate of the dynamic equilibrium of the tautomerism leading to racemization is proportional to the base (Yuchun et al., 2000). Simultaneously, the amount of ($S$)-ester in the system is decreasing due to the hydrolysis with a ($S$)-specific lipase. The shift of equilibrium can be explained using the Le Chatelier's principle where the position of equilibrium will move to a direction so that the concentration of ($R$)-ibuprofen ester will decrease. Hence, the enolate formed will convert the ($S$)-ibuprofen ester and consequently reduce the uncompetitive inhibitor in the system.

**CONCLUSION**

The increasing popularity of chirality in pharmaceutical activity has stimulated an increasing demand for economical and high productive methods for commercial synthesis of pure enantiomers. The demand for those optically pure therapeutic agents is becoming more stringent due to its more target-specific therapeutic effect than racemic mixtures. The optically active ($S$)-ibuprofen acid crystal could be obtained via the proposed process. The use of enzymatic biphasic membrane reactor for dynamic kinetic resolution becomes more potent and viable as the innovative technology contributed to the achievement of 100% theoretical yield when combined with racemization process.

**ACKNOWLEDGEMENTS**

The authors would like to acknowledge Universiti Sains Malaysia (USM) for providing research facilities and grant (USM-RU 1001/PJKIMIA/814114) which resulted in this article.

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